Posters

Protein-Ligand Interactions II

2846-Pos Board B1
Volumetric Characterization of Tri-N-Acetylglucosamine Binding to Lysozyme
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Volumetric characteristics of protein recognition events determine the direction of pressure-induced shifts in the recognition reaction, while also providing insights into the structural, dynamic, and hydration changes. We report changes in volume, αV, and adiabatic compressibility, αKS, accompanying the binding of tri-N-acetylglucosamine [(GlcNAc)3] to lysozyme at 25 °C in a pH 5.5 sodium acetate buffer. We interpret our measured changes in volume and compressibility in terms of changes in hydration and dynamic properties of the protein. Based on our αV data, we find that 79 ± 44 water molecules are released to the bulk from the hydration shells of the protein and the ligand. Our αKS data suggest a 4 ± 2 % decrease in the mean-square fluctuations of the intrinsic volume of the protein, $<\delta VM2>$ (or a 2 % decrease in δVM). Thus, the trisaccharide-bound state of the enzyme is less hydrated, more rigid, and less dynamic compared to the unbound state. In general, we discuss the importance of volumetric insights into the molecular origins of protein recognition events.

2847-Pos Board B2
X-Ray Single Molecule Tracking of MHC/Peptide Complex Reveal a Novel form of TCR Recognition Pattern
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Class II MHC can take loosely bound peptides conformations, which generated in the recycling endosomes or cell surface, that are recognized by autoimmune T cells. H2-DM converts loosely bound complexes to tightly bound complexes; however the mechanism of H2-DM action is not solved yet. The loosely bound peptide/MHC complex may have larger flexibility than tight one. We tried to detect such flexibility of peptide/MHC complex as well as H2-DM function by examining the Brownian motion of peptide/MHC complex with diffracted X-ray tracking (DXT) that monitors real-time movements of individual peptides/MHC in solution at the single-molecule level. We used I-A^k and peptides that differ in their length. Those peptides share same MHC binding sequence, however longer peptide has extra four amino acids at the N-terminus; consequently longer peptide has higher affinity than shorter one for I-A^k. DXT revealed that the motion of long-peptide/MHC complexes cease upon incubation by themselves, whereas the motion of short-peptide/MHC complex sustain the original state for days. H2-DM acts on both complexes and diminishes the motion instantly. These changes of Brownian motion may reflect the conformational change of MHC. Thus, we suggest that measuring the motion of peptide at milliseconds level presents new parameters for both peptide/MHC complex and H2-DM function.

2848-Pos Board B3
Correlated Motions in Protein Crystals Measured by THz Microscopy
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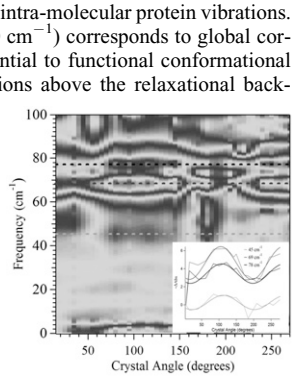
We introduce a new technique, Crystal Anisotropy Terahertz Microscopy (CATM) which directly measures correlated intra-molecular protein vibrations. The terahertz (THz) frequency range (5-100 cm^{-1}) corresponds to global correlated protein motions proposed to be essential to functional conformational changes [1, 2]. CATM accesses these motions above the relaxational background of the solvent and residue side chain librational motions. We demonstrate narrowband features in the anisotropic absorbance for hen egg-white lysozyme (HEWL) single crystals as well as HEWL with triacetylglucosamine (HEWL-3NAG) inhibitor single crystals. By self-referencing to a single orientation, the absorption spectra were obtained and are free from absorption due to librational motions from biological water and artifacts due to multiple reflections within the crystal. The most prominent features for the HEWL crystals appear at 45 cm^{-1}, 69 cm^{-1}, and 78 cm^{-1} and the strength of the absorption varies with crystal orientation relative to the THz polarization. Similar anisotropic features appear in molecular mechanics calculations suggesting specific correlated mode identification is possible, giving us direct insight to the role of correlated motions in conformational changes necessary for ligand binding and catalysis. This work supported by NSF MRI^2 grant DBI295998.



2849-Pos Board B4
Conformational Characterization of Phosphorylated Analogs of a Peptide Mimetic of the Fourth Cytoplasmic Loop of CB1Cannabinoid Receptor
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Phosphorylation is an important regulatory mechanism in receptor signaling. It has been shown that phosphorylation of the CB1 receptor disrupts modulation of ion channels by the receptor and CB1 cannabinoid receptor intracellular C-terminal tail domain (amino acids 401-417) is critical for G(i/o) protein coupling. Further, the proximal portion of the C-terminus of the cannabinoid CB1 receptor is a primary determinant for G-protein activation. The synthetic peptide fragment of the C-terminal juxtamembrane region (CB1 401-417) referred here as IL4 peptide mimicked the receptor's response of inhibiting adenylate cyclase. In the present study, we have used phosphorylated analogues of IL4 peptide to analyze the effect of phosphorylation on the conformation of the peptide and G-protein activation using NMR Spectroscopy. Unambiguous proton NMR assignments have been carried out with the aid of correlation spectroscopy (DQF-COSY and TOCSY) experiments and nuclear Overhauser effect spectroscopy (NOESY and ROESY) experiments. The distance constraints obtained from the NMR data have been used in torsion angle dynamics algorithm for NMR applications (DYANA) to generate a family of structures which have been refined using restrained energy minimization and dynamics. The conformational range of the phosphorylated IL4 peptide revealed by NMR studies has been analyzed in terms of characteristic secondary structural features. The results obtained provide insight into the mechanism by which the peptide activates G-proteins, as a first step in signal transduction.

2850-Pos Board B5
Characterization of the Intramolecular Interactions in the Circularly Permuted GTPase Nucleostemin
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Nucleostemin is a unique protein that plays a role in cell cycle regulation, cellular stress sensing, telomere maintenance, and tumor suppression. Similar to other nucleolar proteins, compartmentalization plays a large role in regulating the activity of NS. The mechanism behind this redistribution is poorly defined but it is linked to the GTPase properties of the protein and an uncharacterized inhibitory region that directs complex formation between NS and various cellular partners. We have been studying the structural and biochemical properties of drosophilia NS1 to better understand the molecular basis of this dynamic localization process. The canonical GTPase core contains a six-stranded β sheet surrounded by 5 α helices. The nucleotide-binding site of the protein is defined by four conserved amino acid sequence motifs, the G-1 through G-4 boxes. cpGTPases are characterized by a reordering of the characteristic G boxes specifically to a G4-G1-G3 pattern, thus a circular permutation of the classic GTPase arrangement. There is little known about how altering the positioning of the G-boxes affects the nucleotide binding and regulatory properties of the protein. Work from our group and other laboratories demonstrated that cpGTPases have broad substrate specificity and can hydrolyze GTP and ATP. We have also identified several structural domains in dNS1 using limited proteolysis. Truncation constructs have been made to elucidate the role of each domain and how the physical interactions between these domains contribute to the biological properties of the protein. Taken together, these data point to a mechanism of action that differs from the larger GTPase family.

2851-Pos Board B6
Relative Promiscuity Between Bacterial and Human Fad Synthetase: A Potential Source of Biomarkers
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FAD Synthetase (FADS) is a ubiquitous enzyme responsible for synthesizing flavin adenine dinucleotide (FAD) by the adenylation of flavin mononucleotide (FMN) using adenosine triphosphate (ATP). In bacteria, this enzyme is bifunctional, in that it catalyzes both the phosphorylation of riboflavin to produce flavin mononucleotide (FMN) and the adenylylation of FMN to produce flavin adenine dinucleotide (FAD). In contrast, the eukaryotic FADS enzyme is